REMARKS

1. General Matters

1.1. At the time of the office action, claims 1-11, 13 and 15-44 were pending, and claims 5, 7, 10 and 19-44 were examined and rejected.

By this amendment, claims 1, 4, 5, 6, 8, 9, 11, 13, 15, 17, 22-25, and 27-33 are amended, and claims 26, 34-44 are cancelled. The T cell epitope limitation of amended claim 5 is based on P19, L7 ("cellular immune response").

1.2. Page 30 has been amended to clarify the meaning of the abbreviation "IPTG". (OA §10). "IPTG" denotes isopropyl-beta-D-thiogalacto sylpyranoside.

1.3. In response to OA §9, we have reviewed the specification to identify any sequences which are explicitly recited but not identified by "SEQ ID NO:". The specification has been amended accordingly.

Also, in response to OA §11, we have amended claims 27, 28, 29 and 30, to insert SEQ ID NOs. No new SEQ ID Nos were needed. The objection to claims 34, 36, 37, 38 and 44 is moot as those claims have been cancelled.

2. Definiteness (OA §13)

2.1. Claim 26 has been cancelled.

2.2. Claims 31 and 32 refer to the figures because that is the most concise manner of identifying the residues in question. While the PTO may have a preference that claims not refer to the drawings, it is not prohibited. The claims of 132 post-1975 patents refer specifically to "figure 1". The residues in question are at the following positions of Omp4:

Omp4: position 568 (Fig 8G): Omp4-Omp7, Omp10-Omp11, Omp13 and Omp15
Omp4: position 572 (Fig 8G): Omp4-Omp11 and Omp13
Omp4: position 587 (Fig 8G): Omp4-Omp11 and Omp15
Omp4: position 608 (Fig 8G): Omp4-Omp11 and Omp15
Omp4: position 635 (Fig 8H): Omp4-Omp11 and Omp15

Omp4: position 767 (Fig 8I): Omp4-Omp12 and Omp15
Omp4: position 875 (Fig 8J): Omp4-Omp12 and Omp15.

2.3. The rejection relative to claim 43 (OA p. 13) is moot as that claim was cancelled.

3. New Matter (OA §14, new)

We have been asked to identify the descriptive support for "said protein being free of any other chlamydial protein". We do not use that exact language. However, we can point to the following:

A. We expressly contemplate vaccine use (see, e.g., P3, L33; P18, L21-24; P19, L10-P20, L18), which implies that the protein must be purified sufficiently to be pharmaceutically acceptable.

B. We teach recombinant expression of the protein (P8, L21-22); P11, L24-26; P20, L19-28). If the host cells are not infected with Chlamydia, they will inherently be free of chlamydial proteins other than that encoded by the deliberately cloned gene.

C. We disclose kits comprising "one or more" of the proteins of SEQ ID NO:2-24. The "one" implies that in some embodiments, the kit provides only one of the twelve recited proteins, implying exclusion of the other 11.

4. Prior Art (OA §6-8, maintained)

The Examiner maintains the rejection of claims 5 (§6), 7 (§7) and 8 (§8) as anticipated by Melgosa. The Examiner asserts that Melgosa teaches "isolated proteins"; "Melgosa isolated the proteins by extracting of outer membrane proteins from elementary bodies and then the purified proteins were separated by electrophores is (SDS-PAGE) see page 300".

The first issue is whether the term "isolated" denotes "complete" purification or "at least some" purification. The term is often suggested by examiners so that a protein or DNA

claim does not inadvertently read on a "product of nature". For this purpose, it need only connote "at least some purification". We have assumed that "isolated" in claim 5, 7 and 8 has this broader meaning.

The Examiner does not directly address whether Melgosa is distinguished by the limitation "free of any other chlamydial protein", see p. 6 of our last amendment. Presumably, the Examiner believes (incorrectly) that he can discount this limitation if he rejects it as new matter (see OA §14).

However, that is plain error. The Examiner's attention is respectfully directed to MPEP §2173.06: "All words in a claim must be considered in judging the patentability of a claim against the prior art... The fact that terms may be indefinite does not make the claim obvious over the prior art". The principle appears equally applicable to terms questioned for lack of description. The Examiner must

(1) explain how Melgosa would satisfy the claim <u>with the challenged limitation</u>, or

(2) withdraw the rejection over Melgosa.

If, later, Applicants choose to excise the questioned phrase to overcome the description rejection, the Examiner may reinstate the Melgosa rejection. In the meantime, the questioned phrase, like any term in a claim, must be given effect.

When this is done, it is clear that Melgosa does not anticipate. While Melgosa thought he had a single 98 kDa protein, he was clearly mistaken. With the last amendment, we attached a copy of Vandahl, et al., Electrophoresis, 22:1204-23 (2001). This shows that several <u>C. pneumoniae</u> proteins have apparent molecular weights of about 98 kDa. If we assume that such determinations are made with an accuracy of ± 10%, then the 98 kDa band includes several proteins of apparent MWs of 89-107 kDa, notably Pmp2, Pmp6, Pmp7, Pmp8, Pmp10, Pmp11, Pmp13, Hypothetical protein Ct 456, Clp proteinase ATPase, and

ClpC protease. Certainly, Melgosa did not isolate any chlamydial protein such that it was free of other chlamydial proteins.

In support of the instant claims, applicants submit declaration (I) of Svend Birkelund. In his declaration, Dr. Birkelund explains that Melgosa did not isolate the 98 kDa protein from the outer membrane complex, but rather merely showed that it was present.

Moreover, according to the declarant, Melgosa was not aware that the 98 kDa band was a mixture of proteins. Such a mixture cannot be sequenced directly. Since Melgosa did not know that a mixture was present, Melgosa did not have motivation to isolate the 98 kDa protein from the mixture. Thus, Melgosa does not render the claims obvious. See MPEP §2143.01.

5. Enablement (OA §5 maintained)

The Examiner maintains the enablement rejection vis-a-vis variants and subsequences. However, claim 5 has now been amended, without prejudice or disclaimer, to excise coverage of variants. Hence, the issue is whether it would have required undue experimentation for the skilled worker to identify a "non-naturally occurring or isolated... peptide or protein which consists of an amino acid sequence, at least 6 amino acids in length, of at least one of said isolated proteins, said... subsequence comprising at least one epitope of at least one of said isolated proteins".

At the top of page 6 of the office action, the examiner remarks "it seems that the applicants argue utility. The rejection was not a utility rejection but [rather] a scope of enablement [one]".

The enablement requirement has two parts, "how-to-make" and "how-to-use". The Examiner cannot seriously dispute that given the disclosure of DNA and AA sequences, and the general reference to recombinant DNA techniques, the skilled worker

could make any desired variant or subsequence as claimed without undue experimentation.

Our remarks concerning use were not directed to utility under §101, but rather to "how to use" under §112. Pages 5-8 of the amendment addressed how one skilled in the art would identify proteins that could be used diagnostically. As we pointed out, diagnostic use requires only retention of immunological activity. Yet the examiner continues to insist that our teaching extend to retention of "the biological activity of the intact protein", and see also the last paragraph of p. 6.

The application expressed the expectation that epitopes, corresponding to subsequences of the identified chlamydial proteins, will be identified. P17, L30-33. An epitope is a structure which is recognized by an antibody (in which case it is a "B cell epitope") or by a T cell (in which case it is a "T cell epitope"). The T cell epitopes include both epitopes recognized by cytotoxic T cells (CD8+) and those recognized by helper T cells (CD4+).

Algorithms have been devised for predicting the location of epitopes, especially B cell epitopes.[1] However, epitopes can be readily identified experimentally, by systematic studies conventional in the art, without resort to such analysis.

A longstanding method of experimentally identifying epitopes is by preparing fragments of the protein and testing the fragments for immune recognition (by antibodies or T cells). Initially, this was done by enzymatic treatment of the protein, which limited the size and variety of fragments which could be obtained in a controlled manner. However, if

[1] Potential short linear B-cell epitopes of Omp4-15 were predicted by means of the GCG suite version 8.1 programs "peptide structure" and "plotstructure", with particular reference to the antigenic index of Jameson-Wolf. However, none of these predicted epitopes were synthesized and tested by applicants.

the amino acid sequence of the protein was known, then recombinant DNA techniques made it possible to prepare any desired fragment.[2] If the desired fragment were reasonably small, it could alternatively be prepared by chemical means (e.g., Merrifield synthesis).

It thus became possible to "sample" the entire protein for the sites of antigenic activity, i.e., to prepare and test fragments which collectively spanned the entire protein. However, use of "abbutting" fragments would run the risk of missing an epitope at a junction of two fragments. Geysen solved this problem by systematic testing of overlapping fragments. See Geysen, et al., USP 4,708,871; WO84/03564, J. Immunol. Meth., 102:259-74 (1987), Science, 235:1184-90 (1987) (copies enclosed with July 8, 2003 amendment).

For the identification of B-cell epitopes, it became customary to synthesize fragments, each 6-10 a.a., with extensive overlapping. Thus, the fragments might be AAs 1-6, 2-7, 3-8, 4-9, 5-10, etc. of the protein, which is a library of hexapeptides with 5 a.a. overlap.

The same technique has also been used to identify cytotoxic (CD8) and helper (CD4) T cell epitopes. For example, Wallace, et al., J. Virol. 65(7) 3821-28 (1991) identified two CD4+ T-cell epitopes on Epstein-Barr Virus glycoprotein gp340 by screening overlapping 15 mer peptides with a 12 a.a. overlap (e.g., 1-15, 4-18, 7-21, etc.). Strong peaks were observed for peptides 6781 and 163-177. Likewise, Petersen, et al., Infect. & Immun., 60:3962-70 (1992) identified CD4+ T-cell epitopes on pertussis toxin 54 subunits by screening overlapping 25- or 26-mer peptides with a 15 or 16 a.a. overlap.

As explained in the attached Birkelund Declaration, Applicants prepared seven overlapping fragments of Omp11

[2] DeMotz, et al., J. Immunol., 142:394-402 (1989) identified T cell epitopes (class II) within proteolytic and recombinant fragments of tetanus toxin.

(Pmp8) and tested them for activation of lymphocytes. One, Fragment 3, was shown to be active.

Applicants then further fragmented fragment 3 into a series of 30 mer peptides with a 10 a.a. overlap. One 30 a.a. fragment, corresponding to AAs 377-406 of Omp11 (Pmp8) (SEQ ID NO:16), was identified as comprising a CD4+ T-cell epitope. This peptide was then shown to be immunoprotective in mice.

Thus, Applicants have obtained a fragment which satisfies the claim limitations.

An important distinction between B cell and T cell epitopes is that B cell epitopes are associated with the folded, biologically active protein, and T cell epitopes with protein fragments displayed by Class I (CD8) or Class II (CD4) MHC molecules.

This means that B cell epitopes can be linear or conformational. Linear epitopes are those defined by a short contiguous subsequence of amino acids, and are preserved if the protein is denatured, or fragmented into a fragment retaining the entire epitope. Conformational epitopes are those which are peculiar to a particular folded conformation, and may comprise amino acids which are not contiguous within the sequence but which are brought into proximity as a result of protein folding.

Applicants believe that the conformational B cell epitopes of Omp4-15 are immunodominant in chamydia-infected subjects. See, e.g., P5, L18-36; P32, L22-26; and Pedersen, et al., Microbiol. Lett., 153 (2001), especially page 154, col. 1 (copy enclosed). Consequently, they did not have motivation to synthesize and test potential linear oligopeptide B cell epitopes such as those given high scores by the Jameson-Wolf algorithm. For the same reason, Applicants have amended claim 1 to cover only those subsequences of Omp4-15 which comprise T cell epitopes.

Applicants have made a very limited attempt to identify CD8+ T cell epitopes (haplotype H-2Db or H-2Kb in C57/Black

mice) in the proteins Omp4, 5, 11 and 12. They synthesized two 9-11 mers from Omp5 and Omp11, and one each from Omp4 and Omp12.[3] None were recognized by the tested CD8+ T cell lines.

Respectfully submitted,

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Enclosures
-Pedersen et al. (2001)

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[3] The Omp4 sequence FVLSNPHPL (782)and the Omp5 sequence LAWTNTGYL (593) have the anchor residues (N at 5, M, I or L at 9) characteristic of H2-Db nonamers, the Omp11 sequence INQAFTQPL (536) has the F-5 anchor of H2-Kb nonamers, and the remaining peptides SIDLFSL (Omp12), TFTGFSNL (omp11, 120) and ISFAFCQL (Omp5, 683) have the F-5 and L-9 anchors of H2-Kb octamers. The presence of the anchor residues, by themselves is not sufficient to guarantee activity.